DIAGNOSIS OF DEMYELINATING OR SPONGIFORM DISEASE

This invention relates to the diagnosis of demyelinating diseases and spongiform encephalopathies in animals and humans, especially BSE and similar or related diseases in humans.

In our co-pending International application WO97/02667 we have disclosed a new diagnostic test for spongiform encephalopathy and other demyelinating conditions in mammals. The test disclosed in our prior application is based on a model of the genesis of this pathological state which is applicable to the various forms in which it is manifest in humans and other animals. In relation to the bovine spongiform disease this model provides an alternative to the current theory based on the formation of prions. Briefly, the new model is based on the phenomenon of molecular mimicry according to which mammals exposed to certain bacteria having peptide sequences which mimic myelin peptides experience an auto-immune reaction. Foremost among the bacteria that are involved in the induction of the auto-immune reaction are Acinetobacter species, especially Acinetobacter calcoaceticus. The diagnostic test based on the new model opens up the possibility of early treatment of these infections e.g. by use of an appropriate antibiotic to prevent further auto-immune attack on the animal's own myelin.

In our International application WO99/47932, we have confirmed the presence of elevated levels of Acinetobacter IgA antibodies in sera of patients suffering from multiple sclerosis (MS) and Creutzfeld-Jacob disease CJD.

In our priority UK application 9825948.4 we described further tests which confirmed the presence of antibodies to bovine myelin and also to bovine neurofilaments in the sera of cows that have died from BSE. These antibodies are of the IgA type. Similar results have also been obtained with sera from patients suffering from MS and CJD. These findings confirm the validity of the model described above and permit the conclusion that we have discovered a general pattern of the origin of similar diseases that occur or may

occur in vertebrates including humans and other farm animals e.g. in poultry farms. Our latest results also provide the basis of a further test for the early identification of these diseases, especially incipient BSE in cows. This further test may either be alternative to or additional to that based on the detection of IgA antibodies to Acinetobacter species e.g. Acinetobacter calcoaceticus.

The present invention therefore comprises a method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies, especially IgA antibodies, which bind to myelin and/or neurofilaments or antigenic (immunogenic) parts thereof, including peptide components as hereinafter specified.

The method preferably comprises assaying for antibodies to myelin and /or neurofilaments of vertebrate species e.g bovine or human species. However, myelin and neurofilaments from other species which are sufficiently homologous to those of bovine or human species to bind to the antibodies under estimation may alternatively be used .

In carrying out the method a positive result is indicated by levels of antibodies at least about two standard deviations above that of control samples.

The invention also comprises a diagnostic kit for the detection of spongiform disease or demyelinating disease in vertebrates comprising, as test antigen, myelin or neurofilaments or antigenic (imunogenic)parts thereof.

Sub. B1

~~The test antigen used in the above defined method and diagnostic kit may~~ be a peptide component of the myelin or neurofilaments, such as one of the following peptides having Sequence ~~ID Nos 1-8~~, namely,

1. NEALEK 2. LKKVHEE 3. EALEKQL 4. ELEDKQN

~~2. EALEKQL 6. KKVHEE 7. EIRDLR 8. EQEIRDLR~~

~~The above sequences have been retrieved from the Protein Information Resource database release 44.~~

Sub. B1

In view of the greater specificity of the IgA antibodies in the immune response it may be concluded that the mechanism of infection with Acinetobacter is via the mucous membranes of the body, the primary sites being the gut or the nasal passages. It is possible that the nasal passages are the site of infection, resulting from inhalation of dust formed from dried sewage or animal excrement and carrying Acinetobacter. The knowledge of this mechanism implies the need for improved hygiene practices in the rearing of farm animals.

Experimental

Assays for the above mentioned organisms are described in our co-pending applications identified above, the contents of which are hereby incorporated by reference. Similar assay procedures using myelin protein or neurofilaments as test antigens are described below.

ELISA TEST:

(1) Aliquots of 200ul of the antigen suspension A or B were absorbed on 96 well flat bottomed rigid polystyrene microtitre plates overnight at 4 deg. Cent. (Antigen A is bovine myelin from Sigma Chemical Company, Fancy Road, Poole, Dorset, BH12 4XA, UK, at a concentration of 5ug/ml and antigen B is bovine neurofilaments from Sigma also at a concentration of 5ug/ml).

(2) The plates are then washed 3 times with phosphate buffered saline (PBS) 0.1% (v/v) Tween 20.

(3) Aliquots of 300ul of blocking solution (0.2% w/v ovalbumin, 0.1% v/v Tween) in PBS is added to each well and incubated for one hour at 37 deg. Cent.

(4) The plates are then washed 3 times with PBS. Tween 20.

(5) Aliquots of 200ul serum samples (test or control) diluted 1/200 in PBS. Tween is added and incubated for 2 hours at 37 deg. Cent.

(6) The plates are then washed 3 times with PBS. Tween 20.

(7) Aliquots of 200ul of peroxidase conjugated rabbit anti-cow IgA (alpha chain) diluted 1/4000 with PBS. Tween are added and incubated for 2 hours at 37 deg. Cent.

(8) The plates are then washed 3 times with PBS. Tween 20.

(9) The development of the colorimetric assay takes place at room temperature for 20 minutes, after the addition of 200ul per well of 0.5 mg/ml (2,2'-azinobis (3-ethylbenz-thiazoline-6-sulphonic acid) in citrate/ phosphate buffer, pH 4.1, containing 0.98 mM hydrogen peroxide.

(10) The reaction is then stopped with 100ul of 2 mg/ml sodium fluoride and optical densities measured at a wavelength of 630 nm with a micro-ELISA plate reader.

(11) All assays are done under coded conditions, in that the tester is unaware of the origin of the serum being studied (Test or control).

(12) All tests are done in duplicate.

The foregoing test procedure may be carried out in the same manner using human myelin or neurofilaments or peptides derived therefrom.

This assay is a novel way of diagnosing cattle suffering from bovine spongiform encephalopathy and humans suffering from MS and CJD in that it describes a test where antibodies to two brain antigens can be determined in bovine or human sera. Any reading in excess of 2 standard deviations of the healthy controls would indicate a positive response. Furthermore the test should be positive (above 2 standard deviations) for both antigens: (A) Bovine myelin protein and (B) Bovine neurofilaments.

This is the first assay that describes measurements of autoantibodies to brain antigens in BSE affected cattle and patients with MS and CJD.

Results for BSE are shown in the accompanying Figures 1 and 2.

Those for MS and CJD are shown in the accompanying Figure 3.

The tests described in our above-mentioned International applications may be combined with that of the present invention. This combined test is particularly suitable for use in testing for BSE. This combined test may be termed the "MAN test" and is based on separate measurements of autoantibodies to bovine myelin (white matter of the brain) and to bovine neurofilaments (gray matter of the brain), as well as to specific antibodies to the saprophytic bacterium Acinetobacter calcoaceticus.

The auto-antibodies to bovine myelin and to bovine neurofilaments and antibodies to Acinetobacter are measured as previously described, for each animal tested. The MAN index is then obtained by multiplying the optical densities according to the following algorithm: =

Myelin IgA autoantibody x Acinetobacter antibody x Neurofilaments autoantibody i.e. the multiplication product M x A x N.

The accompanying Figure 4 shows the results of this test when compared to healthy "organic"controls or to controls (CVL) suffering from other diseases. (CVL = Central Veterinary Laboratory, UK, from where these sera from animals with other diseases were obtained).

The MAN test is calibrated against "organic" farm controls, that is animals coming from a farm where the feedstuffs consist of grass and hay only. The MAN test is an empirical test, in that very low values are obtained for the MAN index, when healthy cows only are tested.

A positive response is recognised when the MAN index is 3 standard deviations above the value found in controls, when testing the serum of a cow suspected of having BSE.

Inv. a'